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Bacteriological analysis of drinking water by using MPN method

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Tamilvani.T and Tamilmathi.S

PG and Research Department of Microbiology, Sengamala Thayaar Educational Trust Women's College, Sundarakkottai, Mannargudi - 614 016, Tamil Nadu, India.

Abstract

Water is extremely essential for survival of all living organisms. Over one billion people worldwide have no access to safe drinking water.Consumption of drinking water contaminated with fecally originated pathogenic bacteria is mostly responsible for the onset of water borne disease outbreaks especially in developing countries.The quality of potable water and treatment of waterborne diseases are critical public health issues. Bacterial contamination of drinking water sources is the most common health risk. Bacteriological analysis of drinking water from three different regions of Thiruthuraipoondi ,Thiruvarur District, Tamilnadu, India, were analyzed by multiple tube fermentation test to find the total coliform count, whose results were expressed as most probable number (MPN) index. The parameters such as pH, turbidity, total dissolved solids (TDS) and conductivity were studied. The recorded values were within the acceptable limits of World Health Organization (WHO) guidelines. Three samples out of five were found to be non-potable as they had been contaminated with *Escherichia coli* indicating the risk for fecal contamination responsible for disease outbreaks. Other contaminating Gram negative bacteria were characterized as *Staphylococcus* spp., and *Pseudomonas* spp. The presence of these bacteria was detected in the three samples in the range of 35×10^5 to 97×10^5 CFU / ml. The results suggest that the water tested sources were not safe for drinking. Hence, it is suggested that regular disinfection of these drinking water sources becomes very essential.

Key words: Coliforms, Electro Conductivity Potable, Most probable number, TDS, water, WHO.

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INTRODUCTION

Water is one of the most important elements for all forms of life. It is indispensable in the maintenance of life on earth. It is also essential for the composition and renewal of cells. Despite of this, human beings are continuing to pollute water sources resulting in water related illnesses (Ethiopian Federal MOH, 2004, WHO, 2008).Drinking water plays an important role in the uptake of essential minerals, and the elevated level of non essential elements can cause morphological abnormalities, reduce growth, increase mortality and mutagenic effects (Abera,., et al. 2011).Fecal coliforms have been seen as an indicator of fecal contamination and are commonly used to express microbiological quality of water and as a parameter to estimate disease risk. Most Portable number (MPN)) is a typical test for fecal coliform (Mengesha et al., 2004). Water may also play a role in the transmission of pathogens which are not faecal excreted. Contamination of drinking water with a type of *Escherichia coli* known as O157:H7 can be fatal. Many microorganisms are found naturally in fresh and saltwater (WHO, 1983; Amira, 2011).

Physico-chemical parameters of water are important to determine the quality of drinking water. As per the guidelines of WHO (1996) the physical parameters that are likely to give rise to complaint from consumers include colour, taste, odour and turbidity, while low pH causes corrosion and high pH results in taste complaints (Chan et al., 2007).Presence of coliforms and other pathogens in treated drinking water could be due to ineffective or poor application of water treatment techniques (McFeters et al., 1986; Kamal et al., 1999; Mead et al., 1999; Subramania, 2004; Nahar et al., 2011; Acharjee et al., 2013).

The present study was to investigate the drinking water quality of various sources at different locations of Thrithuraipoondi,Thiruvarur District, Tamil Nadu,India. Non Potable or not by detecting the indicator bacteria through MPN technique as well as quantifying the load of pathogenic bacteria present in the samples.

MATERIALS AND METHODS

Sample Collection

Three drinking water samples were collected from Thiruthuraipoondi ,Thiruvarur District . 50 ml of water sample was collected asepticallty in sterile plastic bottles (Acharjee*et al*., 2013) at each sampling site.

*Corresponding Author :
email: *tamilmicro89@gmail.com*

Analysis of physico chemical parameters

The physico-chemical parameters such as PH, EC , Total dissolved solids(TDS), color and Turbidity of water samples were evaluated to check the quality of drinking water .The TDS content was determined using hand refectrometer .ThePH was determined using digital PHmeter and the EC was determined using the the EC meter (Model no EQ 610).

Qualitative analysis through MPN method

Presumptive test:(Cappuccino and Sherman, 1996).

Presumptive test is the very first portion of the MPN test method. This is primarily done for detection of the Gram negative coliform bacteria in the water samples. For this task, 15 series of test tubes containing 10 mL of lactose fermentation broth were used for each sample. Ten mL, 1 Ml and 0.1 mL samples were added sequentially in 5 test tubes containing 10 mL lactose fermentation broth 2X, 5 tubes containing 10 ml lactose fermentation broth 1X and 5 test tubes containing 10 ml 1X lactose fermentation broth. Each tube was incorporated with Derhum tube indicating gas formation after lactose fermentation by coliform bacteria (Cappuccino and Sherman, 1996).

Confirmed test

The test tubes showing positive results by the accumulation of gas in the Derhum tubes were selected for the confirmation test to determine the presence of *E. coli* in the respective water samples. A loopful of sample from the broths which gave positive result in the presumptive test, were inoculated on EMB agar to detect as well as differentiate *E. coli* and other Gram negative coliform bacteria. The plates were incubated at 37ÚC for 24 hours (Cappuccino and Sherman, 1996).

Completed test

This is the final part of the MPN test procedure which was completed after the confirmation of the indicator bacteria *Escherichia coli* found in the EMB agar medium. The suspected *E. coli* from a single colony of green metallic sheen was introduced into a lactose fermentation broth 1x again for the assurance of the gas production after fermentation of lactose.

Identification of Coliform bacteria

Gram staining was also performed for the confirmation of *E. coli* isolates (Cappuccino and Sherman, 1996). Besides the presence of *E. coli* colony with green metallic sheen, there were also other colonies in the EMB medium which were identified by using standard biochemical methods to find a complete microbiological profile of the drinking water samples (Cappuccino and Sherman, 1996; Alfrad, 2007).

Standard plate count Technique

Coliform level of less than 10 CUF / 100 ml in a given sample is considered to satisfy the bacteriological requirement for potable water. There are many microorganisms commonly present in drinking water whose numbers far exceeds those of the coliform group and that can interfere with the development of coliforms. The standard plate count was also known as the heterotrophic plate count provides an index of the level of this general bacterial population. This count could be used for quality control in water treatment plant and as a measure of quality deterioration in wells , distribution lines and reservoirs (APHA, 2005).

Isolation and Enumeration of Microorganisms

These methods are grouped on two aspects. One measures the rate at which bacteria carrying particles are settling by gravity from the water on exposed surface,and in the second method the number of bacteria and coliform groups present in the water samples are counted. The water sample were serially diluted as per the serial dilution technique. Simultaneously , nutrient agar plate was prepared. Then 0.1 ml of water sample was taken and diluted to 10^{-4} and 10^{-5} dilutions in tubes and inoculated into the nutrient agar plate by spread plate technique using L-glass rod. Then the plates were incubated at 37°C for 24 hrs. From the incubated nutrient agar plate the bacterial and coliform groups were counted. After that,a single colony was picked and streaked on to the selective medium for better isolation of the microorganisms . The same procedure was followed for enumeration of fungi from different dilution tubes such as 10^{-2} and 10^{-3} and the plates were incubated at 37°C for three days or even a week.

Identification of bacterial isolates

The isolated colonies were subjected for the identification of various microorganisms by using Grams staining and biochemical test.Bacterial isolates were identified on the basis of biochemical characteristics .The first step was gram staining using a staining test (Lay, 1994) followd by carbohydrates fermentation test , Indole (Lay, 1994), Methyl (Ijong, 2003) and catalase test (Lay, 1994).

RESULT AND DISCUSION

Water is absolutely vital not only for the survival of human beings, but also for plants, animals and all other living organisms (Razo et al. 2004). Physico chemical of three different drinking water samples, three different sources of tap water were collected from Thiruthuraipoondi region, Thiruvarur (Dt.), were investigated in the present study. The physico-chemical analysis of water supplies is necessary to

Table 1. Physico-chemical analysis of drinking water sample

Water sample	TDS	EC	pH
Sample 1	50	0.23	6.3
Sample 2	52	0.14	5.4
Sample 3	42	0.1	5.3

Table 2. Biochemical characterization of isolated colonies

Biochemical tests	*E.coli*	*Staphylococcus* spp	*Pseudomonas* spp
Shape	Rod	Rod	Cocci
Gram Staining	-	-	+
Motility	Motile and Non-motile	Motile	Non-motile
Indole	+	-	-
Methyl red	+	-	-
Voges Proskauer	-	-	-
Utilization	-	+	+
Triple sugar iron agar Test	A/A	K/A	A/A
Urease	-	+	+
Catalase	+	+	+
Oxidase	-	-	+

Note :- A/A : Acid slant/ Acid Butt, K/A : Alkaline slant/ Acid Butt, K/NC : Alkaline slant/ No change in Butt, (-) : Negative result, (+): Positive result, colony 1: *E. coli*, colony2: *Staphylococcus* sp. and colony 3: *Pseudomonas* sp.

guarantee the quality, compliance with established quality criteria and efficiency of operation of water treatment plants and distribution system. pH is a measure of the balance between the concentration of hydrogen ions and hydroxyl ions in water. The pH of water provides vital information in many types of geochemical equilibrium or solubility calculations (Hem, 1985).

Results revealed that the pH and the temperature of the water in the distribution system fall within the WHO limits. In all the water samples the pH was in the range of 5.6to 6.7 as shown in the Table 1. The Target Water Quality Range (TWQR) for pH in water for domestic use varies from 6 - 9 (DWAF, 1996).

TDS describes the load of inorganic matter and directly relates to conductivity of the water. The TDS for all the water samples were found to be within the desired limit of 50 mg/l as given in the table below (Table 2). According to (Davis and De Wiest 1966), this TDS level of water samples is desirable for drinking purposes. The electric conductivity varied from 96 to 1775 µs/cm, with 406 mean and standard deviation of 233.8 (Table 1). The recorded TDS and conductivity are correlated of Ions from the dissolved solids in water create the ability for that water to conduct an electrical current, which can be measured using a conventional conductivity meter (Singh and Karla, 1975; Lystrom *et al.*, 1978).

Determination of microbial contaminants of faecal origin is a major priority in assessing the quality of drinking water. Results from the analysis indicated the presence of coliform and faecal coliform bacteria in the water sample collected from all the sources, which could be due to the contamination of the soil through leaks/ cracks or due to seepage of sewage water into the supply lines (Imran *et al*., 2009). The MPN determination of coliforms from all the water samples at different sources is presented in Table 3. Results reveal that the coliform levels that most of the collected water samples were found to be in their prescribed limit by calculating the MPN Index per 100

Table 3. MPN Determination of coliforms by Multiple Tube Fermentation

S.No.	Combination of positives	Number of tubes giving positive reaction				95% confidence	
		3 of 10ml Each	3 of 1ml Each	3 of 0.1ml Each	MPN Index/ 100ml	Lower	Upper
1	1-1-0	1	1	0	4	1.5	15
2	1-0-0	1	0	0	2	10	11
3	0-1-0	0	1	0	2	1	10

ml. Results showed that the samples collected from all the sources were identified as *E.coli* sp. , *Staphylococcus* sp,and*Pseudomonas* sp., was identified (Table 2) and this could be due to the improper maintenance of the particular water plant.

High microbial counts in water are undesirable because of the increased likelihood of the pressure of pathogens. The viable count was measured by standard plate count (SPC) technique using nutrient agar and has been recorded as colony forming unit (CFU/ml). In the present study, results reveal that, the water samples were contaminated with microorganisms such as *E.coli* sp., *and Staphylococcus* sp. and in one of the sample, the bacteria was identified as *Pseudomonas* sp., by performing Gram's staining and various Biochemical tests. Whereas no fungal isolates were found in any of the water sample.

CONCLUSION

Considering the drinking water quality status, the present study aimed to assess the current water status of Thiruthuraipoondi ,Thiruvarur , Tamil Nadu, India. It is expected that monitoring results could lead to remedial measures for improving the existing drinking water quality situation. Drinking Water samples from

3 different sources of tap water were collected and analysed. The physico-chemical properties, coliform test and Standard plate count techniques were carried out.

It is concluded that the pH ranges of all the water samples were found to be mildly acidic in nature. The physico-chemical analysis showed that all the water samples from 3 different sources reveal that they were found to be within the desired and permissible limit of WHO standards. The coliform counts and the general bacterial population in the distribution system exceeded the acceptable limit in sample1 and sample 3. This could be due to the improper maintenance of the particular water plant system. Based upon the results of this study it is recommended that regular monitoring of the physico-chemical analysis and the total coliforms in the water distribution system should be carried out to ensure that the water is safe for drinking.

REFERENCE

Abera, S., Zejinudin, A., Kebede, B. 2011. Bacteriological analysis of drinking water sources. *Afr. J. Microbiol. Res*., 5 (18): 2638-2641. https://doi.org/10.5897/AJMR11.218

Acharjee, M., F. Jahan, F. Rahman, and R. Noor. 2013., Bacterial proliferation in municipal water supplied in Mirpur locality of Dhaka City, Bangladesh. Clean – *Soil, Air, Water* 41: 1-8.

Alfrad, E.B.2007. Bacteriological analysis of drinking water.Bensons Microbiological Applications. Mcgraw-Hill Book Company.

Amira, A.A., Yassir, M.E. 2011. *Bacteriological quality of drinking water* in Nyala, South Darfur, Sudan. Environ. Monit. Assess, 175: 37–43. PMid:20480392 https://doi.org/10.1007/s10661-010-1491-7

APHA. 2005. Standard Methods for the Examination of Water and Wastewater. 21st ed. American Public Health Association, Washington DC. P. 46.

Cappuccino, J.G. and Sherman, N. 1996. *Microbiology - ALaboratory Manual*. The Benjamin/Cummings publishing Co., Inc., Menlo Park, California.

Chan,C.L., M.K. Zalifah and A.S. Norrakiah. 2007. Microbiological and physicochemical quality of drinking water. *Malaysian Journal of Analytical Sciences*, 11(2): 414-420.

DWAF 1996. South Africa water quality Guidelines. 7: Aquatic Ecosystems (1st Edn) Department of water Affairs and forestry, Pretoria. *American Journal of Environmental Protection*. 2016, 4 (1): 7-20.

Federal Democratic Republic of Ethiopia Ministry of Health (2004). water supply safety measures extension package. *Addis Ababa,* P. 1-4.

Lystrom, D.G., Rinella, R.A. and Knox, W.D. 1987. Definition of regional relationships between dissolved solids and specific conductance, Susquehanna River Basin, Pennsylvania and New York. *Journal of Research US Geology Survey*. 6(4): 541-545.

Hem, J.D. 1985. Study and interpretations of the chemical characteristics of natural water.

Hoko, Z. 2008 An assessment of quality of water from boreholes in Bindura District, Zimbabwe. Phys. Chem. Earth, Parts A/B/C, 33:824–828. https://doi.org/10.1016/j.pce.2008.06.024

Ijong, F, G. 2003. Uji IMVIC. UraianTeoritis Proses Biokimianya. Laboratorium.

Kamal, M. M., Hansen, A. M. and Badruzzaman, A. B. M. 1999. Assessment of pollution of the river Buriganga, Bangladesh, using a water quality model. *Water Sci. Tech*., 40: 129-136. https://doi.org/10.2166/wst.1999.0104

Lay, W.B. 1994. Microbes analysis in laboratory. Raja GrafindoPersada, Jakarta. Indonesia Mikrobiology HasiPerikanan. FPIK Unsrat. Manado New York.

Meade, J. W. 1998. Aquac. Manag. *CBS Publishers & Distributors, New Delhi, India.* P. 9.

MengeshaAdmassu, Mamo Wubshet , Baye Gelaw, 2004. *Ethiopian Journal of Health Development*, 18(2):112-115. https://doi.org/10.4314/ejhd.v18i2.9946

McFeters, G. A., Kippin, J. S. and LeChevallier, M. W. 1986. Injured coliforms in drinking water. *Appl. Environ. Microbiol*., 51 : 1–5. PMid:3513698 PMCid:PMC238806 https://doi.org/10.1128/aem.51.1.1-5.1986

Mead, G.C . 1989. Processing of poultry. *Elsevier Appl. Sci*., 129.

Mengesha A, Mamo W, Baye G 2004. A survey of bacteriological quality of drinking water in North Gondar. *Ethiop. J. Health Dev*., 18: 112- 115.

Nahar, A., Ahmed, M. M. and Chakraborty, A. 2011. A Quality Analysis of Dhaka WASA Drinking Water: Detection and biochemical characterization of the Isolates. *J. Environ. Sci. Nat. Res*., 4 (2): 41-49. https://doi.org/10.3329/jesnr.v4i2.10133

Nkono, N.A and Asubiojo. 1998. Elementalcomposition Southeastern Nigeria *Journal of Radio anal clear Chemisty*. 27: 117-119. https://doi.org/10.1007/BF02386440

Razo, I., L. Carrizales, J. Castro, B. F. Diaz and M. Moroy. 2004. Arsenic and heavy metal pollution of soil, water and sediments in a semi-arid climate mining area in Mexico. Water, air, *Soil Poll*. 152(1-4): 129-152. https://doi.org/10.1023/B:WATE.0000015350.14520.c1

Singh, T. and Karla, Y.P. 1975. Specific conductance method for In Situ estimation of total dissolved solids. *Journal AWWA*. 67(2): 99-100. https://doi.org/10.1002/j.1551-8833.1975.tb02168.x

Singh, A., and G. A. Mefeters. 1992. Detection method for water borne pathogens. *In* R. Mitchell (ed.), Environmental microbiology. John Willey and sons Inc., New York.

Subramania, B. 2004. Water quality in South Asia. *Asian J. Water Environ. Pol.,* 1 : 41-55.

WHO. Guidelines for Drinking water quality, Vol. 1, 2 and 3, 1983.

WHO (2008). Guidelines for Drinking -water Quality, Third Edition, Volume 1, 2008, Geneva, P. 2-7.